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***Calocera bambusicola* sp. nov. and *C. sinensis* newly recorded from Taiwan**

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ABSTRACT — *Calocera bambusicola* is presented as a new species, based on specimens collected from rotten culms of bamboo (*Pseudosasa usawai*) near a sulfur hot spring on Yangminshan mountain in Yangminshan National Park, Taipei, Taiwan. This new species morphologically resembles *C. sinensis* but differs in its much smaller basidiocarps, narrower basidiospores, much narrower contextual hyphae, and growth on bamboo culms. ITS differences also separate these two species. A key to species of *Calocera* with nodose-septate hyphae is provided, and *C. sinensis* is newly reported from Taiwan.

KEY WORDS — *Dacrymycetaceae*, *Dacrymycetales*, taxonomy

Introduction

The genus *Calocera* (Fr.) Fr. (*Dacrymycetaceae* J. Schröt., *Dacrymycetales* Henn.) is ecologically saprobic, causing a brown rot in wood. McNabb (1965) treated twelve species and infraspecific varieties in his worldwide monograph of *Calocera*, among which only four species possess clamped hyphae. Reid (1974), who treated five *Calocera* species in his monographic survey of British *Dacrymycetales*, included a new species, *C. pallidospatulata* D.A. Reid. Peng et al. (1992) recognized six species of *Calocera* from China, including the newly described species *C. mangshanensis* B. Liu & L. Fan (Liu & Fan 1989) and *C. morchelloides* B. Liu & L. Fan (Liu & Fan 1990). The three species published after McNabb's (1965) monographic work all have simple-septate hyphae. Previous taxonomic study of *Calocera* was based on morphological analyses. DNA sequence analyses have never been used to test species delimitations. So far, only *Calocera cornea* (Batsch) Fr. and *C. viscosa* (Pers.) Fr. sequences have been submitted to the GenBank database (Shirouzu et al. 2009).

In 1999–2001, several specimens of *Calocera* were collected from rotten culms of the bamboo species *Pseudosasa usawai* near a sulfur spring on Yangminshan

mountain in Taipei, Taiwan. Basidiocarps of these *Calocera* specimens are very small and bear nodose-septate hyphae. Morphological and ITS sequence analyses reveal that these specimens represented an undescribed species, *C. bambusicola*, which is described here. *Calocera sinensis*, which resembles the new species, is here reported for the first time from Taiwan.

Materials & methods

MORPHOLOGY — Specimens for this study were collected in Taiwan during 1992–2007 and are deposited at the herbarium of the National Museum of Natural Science of ROC (TNM). For microscopic observations, the sections were stained with 1 % aqueous Phloxine in 5 % KOH. Melzer’s reagent (IKI) was employed to detect amyloidity and dextrinoidity.

PHYLOGENY — DNA was extracted from dried fruiting body tissue or cultured mycelia as described in Wu et al. (2007) from eight strains representing six species (TABLE 1). Both DNA strands were sequenced by ITS1 and ITS4 primers (White et al. 1990). Consensus data from the forward and reverse sequences were matched using BioEdit 7.0.4.1 (Hall 1999). Alignment was performed using Clustal X 1.83 (Thompson et al. 1997) and adjusted by hand also using BioEdit 7.0.4.1. The optimized alignment file was analyzed by maximum parsimony (MP) in PAUP 4.0b10 (Swofford 2003). The analytical parameter preferences are described in Wu et al. (2007). Bootstrap analysis (Hillis & Bull 1993) was performed with 1,000 replicates with random addition sequences to obtain estimates of the reliability of the nodes.

TABLE 1. Taxa used in this study, along with their strain/specimen numbers, origins and GenBank accession numbers.

SPECIES*	STRAIN/SPECIMEN NO.	ORIGIN	GENBANK ACCESSION NO.
<i>Calocera bambusicola</i>	Wu 9910 12	Taipei, Taiwan	FJ195751
<i>C. cornea</i>	Wu 0606 1	Taichung, Taiwan	FJ195752
<i>C. sinensis</i>	Wu 0703 6	Nantou, Taiwan	FJ195754
<i>C. sinensis</i>	Wu 0505 3	Nantou, Taiwan	Wu 0505 3
<i>C. sinensis</i>	JCH 070726	Chiayi, Taiwan	FJ195755
<i>C. viscosa</i>	Wu 9905 30	Taichung, Taiwan	FJ195756
<i>Dacrymyces</i> sp.	FPL8953	USA	DQ205684
<i>Guepinopsis buccina</i>	AFTOL ID 888	Washington, USA	DQ206986

*Taxon names in bold indicate sequences from this study.

Taxonomy

Key to five species of *Calocera* with nodose-septate hyphae

- 1a. Basidiospores 1-septate 2
- 1b. Basidiospores 1-3-septate. 4
- 2a. Basidiocarps tiny, shorter than 1.5 mm; contextual hyphae 1.5–3.3 µm diam.;
basidiospores mostly narrower than 4.3 µm *C. bambusicola*
- 2b. Basidiocarps 3–25 mm high; contextual hyphae 3–7.5 µm diam.;;
basidiospores wider than 4.3 µm 3

- 3a. Basidiocarps large, up to 25 mm high; clamped dikaryophyses lacking
..... *C. fusca* Lloyd
- 3b. Basidiocarps smaller, rarely exceeding 10 mm high; clamped dikaryophyses
present *C. sinensis*
- 4a. Basidiocarps cylindrical, spatulate, petaloid, or palmately lobed;
basidiospores 11–15.5 μm long. *C. guepinoides* Berk.
- 4b. Basidiocarps subulate or fusiform; basidiospores 13.5–17.5 μm
long *C. macrospora* Brasf.



FIG. 1. Basidiocarps of *Calocera bambusicola* (Wu 9910-10). (scale bar = 1 cm).

Calocera bambusicola Sheng H. Wu, sp. nov.

FIG. 1, 2 A–C

MYCOBANK MB 516557

Basidiocarpi gregarii, aurantiaci, simplices, clavati vel cylindrici, 0.1–0.3 mm diametro, ad 1.5 mm alti. Hyphae fibulatae. Cystidia desunt. Probasidia subclavata, 20–35 \times 4–5 μm , deinde bifurcate. Basidiosporae ellipsoidea, 9–12 \times 3.3–4.5 μm , 1-septatae, laeves, tenuitunicatae.

HOLOTYPE Taiwan. Taipei City: Yangminshan National Park, Hsiao-yukeng, 25° 11' N 121° 33' E, alt. 700 m, on culm of *Pseudosasa usawai*, leg. S.H. Wu et al., Oct. 23, 1999, Wu 9910-10 (TNM F9942).

ETYMOLOGY — referring to the habitat of this species.

Basidiocarps gregarious, yellow-orange when fresh, orange-brown when dry, gelatinous-cartilaginous when soaked, corneous when dry, simple, clavate or cylindrical, terete or slightly flattened, unbranched, apically blunt, 0.1–0.3 mm diam., up to 1.5 mm high. Hymenium amphigenous, surface smooth. Context with dense texture; hyphae nodose-septate, colorless, 1.5–3.3 μm diam., walls thin or up to 0.5 μm diam. Cystidia lacking. Probasidia subclavate, 20–35 \times 4–5 μm , becoming bifurcate. Basidiospores ellipsoid, adaxially concave, slightly

curved, colorless, 9–12 × 3.3–4.5 µm, smooth, thin-walled, with one septum when mature, bearing a distinct apiculus, IKI-, germination not seen.

ECOLOGY & DISTRIBUTION — All specimens of *Calocera bambusicola* were collected from culms of *Pseudosasa usawai*, beside a sulfur spring; so far known only from northern Taiwan.

ADDITIONAL SPECIMENS EXAMINED — Taiwan. Taipei City: Yangminshan National Park, Hsiao-yukeng, 25° 11' N 121° 33' E, alt. 700 m, on culm of *Pseudosasa usawai*, leg. S.H. Wu et al., Oct. 23, 1999, Wu 9910-12 (TNM F9943); Dec. 30, 1999, Wu 9912-19 (TNM F10187), Wu 9912-20 (TNM F10188), Wu 9912-24 (TNM F10189); Oct. 30, 2000, Wu 0010-205 (TNM F12263); Jun. 15, 2001, Wu 0106-18 (TNM F13446), Wu 0106-19 (TNM F13447), Wu 0106-24 (TNM F13449).

REMARKS — *Calocera bambusicola* is distinct from other species of this genus in having very tiny basidiocarps and by growing on culms of bamboo. This new species resembles *C. sinensis* in having clamped hyphae and similar basidiospores, but differs in having much smaller basidiocarps, narrower basidiospores, and much narrower contextual hyphae.

Calocera sinensis McNabb, New Zealand J. Bot. 3: 36. 1965.

FIG. 2 D–G

Basidiocarps gregarious, yellow to orange when fresh, orange-brown when dry, gelatinous-cartilaginous when soaked, corneous when dry, simple, cylindrical-clavate or spatulate, rarely branched, apically blunt or rarely pointed, 0.5–1.5 mm diam., up to 8 mm high. Hymenium amphigenous, surface smooth. Context with dense texture; hyphae nodose-septate, colorless, 3–7 µm diam., with 0.5–2 µm thick walls. Cystidia lacking. Hyphidia simple or branched. Probasidia subclavate, 25–40 × 3–4 µm, becoming bifurcate. Basidiospores ellipsoid, adaxially concave, slightly curved, colorless, 9–13 × 4.3–5.5 µm, smooth, thin-walled, with one septum when mature, bearing a distinct apiculus, IKI-, germination by conidia or by germ tubes.

ECOLOGY & DISTRIBUTION — This species grows on both gymnosperms and angiosperms in Taiwan, and causes a wood brown rot. Known from China (McNabb 1965, Peng et al. 1992) and Taiwan (this study).

SPECIMENS EXAMINED — Taiwan. Taoyuan Hsien: Takuanshan, alt. 1,500 m, on log, leg. S.H. Wu & S.W. Chou, 20 Jul 1999, Wu 9907-7 (TNM F9939). MIAOLI HSIEN: Kuanwu-Hsuehchien, Peikenghsi Abandoned Trail, 24° 27' N 121° 03' E, alt. 1800 m, on wood, leg. W.N. Chou, 9 Oct 1995, CWN 01311 (TNM F4397). Taichung Hsien: Anmashan, 24° 16' N 120° 00' E, alt. 2,250 m, on trunk of gymnosperm, leg. S.H. Wu & H.J. Chan, 8 Nov 1997, Wu 9711-30 (TNM F9382); on rotten trunk of angiosperm, leg. S.H. Wu et al., 10 May 1999, Wu 9905-25 (TNM F9937); Tashueishan Forest Road, alt. 1200 m, leg. S.Z. Chen, 31 May 1999, Chen 865 (TNM F9919). Nantou Hsien: Hsitou, 23° 40' N 120° 47' E, alt. 1200 m, on stump of *Cryptomeria japonica*, leg. S.H. Wu, 19 Jun 1993, Wu 9306-11 (TNM F1054), Wu 9306-12 (TNM F1050); on stump of *Cryptomeria japonica*, leg. S.H. Wu, 27 Jul 1993, Wu 9307-78 (TNM F1243); on trunk of *Cryptomeria japonica*, leg. S.H. Wu, 6 Apr 1997, Wu 9704-19 (TNM F8604); on rotten wood of gymnosperm, leg. S.H.

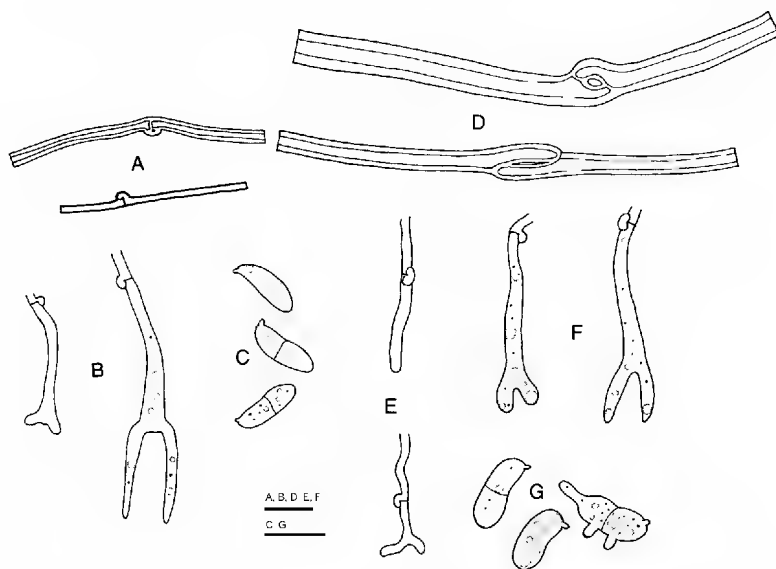


FIG. 2. *Calocera bambusicola* (Wu 9910-10: A-C); *C. sinensis* (Chen 865: D-G). Contextual hyphae (A, D); hyphidia (E); basidia (B, F); basidiospores (C, G). (scale bars = 10 µm).

Wu, 3 May 2005, Wu 0505-3 (TNM F18731); on branch of gymnosperm, leg. S.H. Wu & S.Z. Chen, 6 Jul 2007, Wu 0703-6 (TNM F20958); Sunlinhsi, 23° 38' N 120°47' E, alt. 1700 m, on fallen branch of *Cryptomeria japonica*, leg. S.H. Wu, 1 Jul 1992, Wu 9207-42 (TNM F0272); 19 Sep 1992, Wu 9209-88 (TNM F0361); Yushan National Park, Tatchia, 23° 29' N 120°54' E, alt. 2600 m, on decorticated branch of angiosperm, leg. S.H. Wu, 25 Nov 1993, Wu 9311-106 (TNM F1520). Ilan Hsien: Chilanshan, 24° 41' N 121°28' E, alt. 400 m, on wood of angiosperm, leg. S.H. Wu, 18 Nov 1992, Wu 9211-17 (TNM F0486).

REMARKS — *Calocera sinensis* has only been reported from China (McNabb 1965, Peng et al. 1992). In Taiwan, this species was collected from temperate to subtropical belts and is fairly common. *Calocera fusca* is allied to *C. sinensis*, but differs from the latter in having larger basidiocarps and lacks clamped dikaryophyses. *Calocera fusca* was reported from New Zealand, Australia, and Juan Fernández Islands (McNabb 1965), and China (Peng et al. 1992). However, further study should be performed to evaluate the importance of the features used for separating these two species.

Results and discussion on analysis of the ITS region

Amplification of the ITS region yielded PCR products approximately 530 bp long. The final alignment of the 8 sequences included 533 positions. Excluding the ambiguous sites at both ends, 452 sites were used for the MP analysis. Only

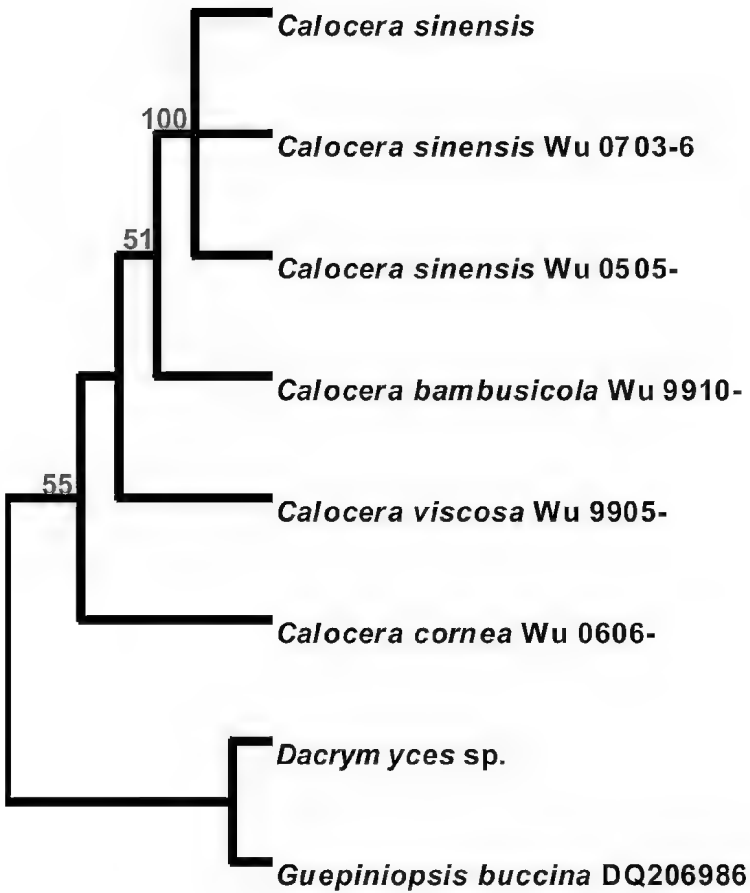


FIG. 3. The most parsimonious tree derived from ITS DNA sequence data. Bootstrap values are shown at nodes supported by no less than 50% from 1000 replicates. TL = 293, CI = 0.846, RI = 0.724.

one most parsimonious tree (293 steps, CI = 0.846, RI = 0.724) was retained in this analysis. Of the 452 included sites, 264 were constant, 96 were variable but parsimoniously uninformative, and 92 were parsimoniously informative. Bootstrap percentages were assigned above the branches. Three strains of *C. sinensis* were clustered together with 100% bootstrap support, and they were further clustered together with *C. bambusicola*, supported by a 51% bootstrap value (FIG 3).

The phylogenetic analysis indicates that the two species with clamp connections (*C. bambusicola*, *C. sinensis*) are more closely allied to each other than to two other species without clamp connections (*C. cornea*, *C. viscosa*). The three studied *C. sinensis* strains are identical in the alignment matrix of the 452 sites, after excluding the ambiguous sites at both ends. However, there were 32 transition, 49 transversion, and 29 deletions between *C. bambusicola* and *C. sinensis*, showing a 23.34% difference between the two species. Our ITS sequence analysis strongly supports *C. bambusicola* as independent from *C. sinensis*.

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